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Bacterial Communities of Bartonella-Positive Fleas in Gut Microbiota of Armenian Populations

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ABSTRACT

Bartonella spp. are known as causative agents of zoonosis. The information on the reservoirs of *Bartonella* spp. mammals /fleas/ ticks, is limited in Armenia. The aim of this study was, on the basis of the available PhyloChip™ data from the previous investigations, to study bacterial communities in healthy and patients with Familial Mediterranean Fever of the Armenian population that have common gene sequences with *Bartonella*-positive fleas.

The preliminary results on PhyloChip™ analysis revealed operational taxonomic units of several gut bacterial communities in healthy people and patients with FMF in the Armenian population sharing common gene-sequences with the *Bartonella*- positive fleas.

Introduction

Bartonella spp. bacteria can be observed around the globe and are the causative agents of emerging and reemerging human diseases. Bacteria of the genus *Bartonella* are fastidious, gradually-growing gram-negative aerobic rods. They parasitize erythrocytes and endothelial cells of a wide range of mammals and are generally host specific at different taxonomical levels (Bai, et al., 2013, Breitschwerdt, 2017, Kosoy, 2018, Ying, et al., 2002) (Table 1).

Rapid growth of populations, particularly in areas with weak health systems, urbanization, globalization and inequalities within cities, climate change, and the changing nature of pathogen transmission between

human and animal populations are an important cause of zoonosis. *Bartonella* species, widely known as causative agents of zoonosis, involve a broad spectrum of clinical syndromes from self-limited cat-scratch disease (CSD) to potentially fatal diseases, such as endocarditis (Chomel, 2009) mild lymphadenopathy and fever (Anderson, et al., 1997, Kosoy, et al., 2010, 2018, Chomel, et al., 2004), and their DNA can be found in multiple vectors (Cheslock and Embers, 2019).

Pets (Chomel, et al., 2006), especially cats (Razgūnait, et al., 2021), are large reservoirs for human infection (Angelakis, et al., 2014, Iannino, et al., 2018). The disease is widely transmitted to cats (Petríková, et al., 2021) and

rodents (Gutiérrez, et al., 2015) by fleas. It has also been discovered in a wide range of mammals, including humans, cats, dogs, rabbits, rodents, horses, cattle, and other wildlife (Chomel, et al., 2009, Iannino, et al., 2018, Breitschwerdt, et al., 2017).

Materials and methods

Historically, the most common causative agents for human cases of bartonellosis have been *Bartonella bacilliformis*, *Bartonella quintana*, and *Bartonella henselae* (Angelakis, et al., 2014, Breitschwerdt, et al., 2019). These infections are characterized by a prolonged intraerythrocytic bacteremia, fever, headache, and malaise to more severe

symptoms such as hallucinations (Ben-Tekaya, et al., 2013, Kalogeropoulos, et al., 2019).

According to retrieved PubMed information, several cases of bartonellosis have been reported in Caucasus (Malania, et al., 2016), but the information on the reservoirs of *Bartonella spp.*: mammals/fleas/ticks, is limited in Armenia. The third generation, culture-independent, high-density DNA microarray (PhyloChip™; Affymetrix, Santa Clara, CA, USA) (Harutyunyan, et al., 2013, 2014a, 2014b, Pepoyan, et al., 2014a, 2014b, 2015a, 2015b, 2018, 2019, 2021, Piceno, et al., 2013) was used during our previous investigations on gut microbiota of Familial Mediterranean Fever (FMF) patients. The aim of this study was, on the basis of the available PhyloChip™ data from the previous investigations, to study bacterial communities in healthy people and patients with FMF in the Armenian population that have common gene sequences with *Bartonella*-positive fleas.

Table 1. The known *Bartonella* species, their hosts and their vectors*

Bartonella Species	Host (s)	Vector(s)
<i>B. henselae</i>	Cat, human, dogs, horses	Fleas, lice, ticks, spiders
<i>B. quintana</i>	Humans, macaques, cats, dogs	Human body lice, fleas, bed bugs
<i>B. bacilliformis</i>	Humans	Sandflies, fleas
<i>B. koehlerae</i>	Cats, dogs, humans	Fleas
<i>B. vinsonii ssp. berkhoffi</i>	Dogs, horses, foxes, humans	Fleas, ticks
<i>B. bovis</i>	Cattle, cats, dogs, human	Biting flies, ticks
<i>B. clarridgeiae</i>	Cats, dogs	Fleas, ticks
<i>B. rattimassiliensis</i>	Rats	Fleas
<i>B. tamiae</i>	Rats, humans	Mites
<i>B. tribocorum</i>	Rats	Fleas
<i>B. rousetii</i>	Bats	Bat flies
<i>B. schoenbuchensis</i>	Cattle	Biting flies, ticks
<i>B. chomeli</i>	Cattle	Biting flies, ticks
<i>B. doshiae</i>	Rats, humans	Fleas
<i>B. grahamii</i>	Mice, humans	Fleas
<i>B. birtlesii</i>	Mice	Fleas
<i>B. mayotimonensis</i>	Bats, humans	Bat flies, fleas, ticks
<i>B. elizabethae</i>	Rats, humans, dogs	Fleas
<i>B. washoensis</i>	Dogs, humans	Fleas, ticks
<i>B. rochalimae</i>	Dogs, humans	Fleas, ticks
<i>B. vinsonii ssp. arupensis</i>	Dogs, humans	Fleas, ticks
<i>B. melophagi</i>	Sheep, humans	Sheep keds
<i>B. alsatica</i>	Rabbits, humans	Fleas, ticks

*The table has been adapted from Breitschwerdt E.B., 2017, Chapter 5.2.

Results and discussions

Forty healthy and forty FMF volunteers participated in investigations. A detailed information of these investigations is available at Pepoyan et al. (Pepoyan, et al., 2018, www.ncbi.nlm.nih.gov).

Also, the literature data were collected from PubMed (1997-2021, main keywords: *Bartonella spp* and Caucasus).

Bacterial communities of *Bartonella*-positive fleas in gut microbiota of Armenian healthy and FMF patients' populations are presented in Table 2. The pilot PhyloChip™ analysis revealed operational taxonomic units (OTU) of several gut bacterial communities in healthy people and patients with FMF in the Armenian population sharing common gene-sequences with the *Bartonella*-positive fleas. According to Table 2, *Proteobacteria*, *Fusobacteria* and *Firmicutes* were the main bacterial phyla sharing common gene-sequences with the *Bartonella*-positive fleas. There were no statistically significant differences in the hybridization scores of these bacteria from the healthy and diseased groups.

The investigations on the seroprevalence against *B. henselae* and *B. quintana* of 536 people from the Eastern Slovakia, with no data on the human bartonellosis cases as it was in Armenia, revealed that 23.5 % of the people demonstrated positivity for anti-*B. henselae* antibodies and 24.8 % against *B. quintana* (Petríková, et al., 2021). Statistically significant, but clinically irrelevant differences in uric acid levels and serum creatinine were described between *B. henselae* seropositive and seronegative groups (Petríková, et al., 2021).

Table 2. Bacterial communities of *Bartonella*-positive fleas in gut microbiota of Armenian healthy and FMF patients' populations*

Bacterial genus	Healthy Men volunteers (N=40)	Male FMF volunteers (N=40) (hybridization score)
<i>Haemophilus</i>	598±105 ^a	432 ± 125 ^a P>0.05
<i>Aquabacterium</i>	4137±760 ^a	5005 ± 1351 ^a P>0.05
<i>Aquabacterium</i>	1408±507 ^a	1970 ± 102 ^a P>0.05
<i>Aquabacterium</i>	3070±885 ^a	3673 ± 189 ^a P>0.05
<i>Faecalibacterium</i>	9642±257 ^a	9268 ± 251 ^a P>0.05
<i>Oscillospira</i>	1930±922 ^a	2623 ± 119 ^a P>0.05
N – Number of volunteers a-hybridization score P < 0.05 is statistically significant		

*Composed by the authors.

Conclusion

Thus, future investigations on reservoirs of *Bartonella* spp. in mammals/fleas/ticks in Armenia, as well as future immunologic and molecular biological investigations on transmission of *Bartonella* spp. are required.

References

- Anderson, B.E., Neuman, M.A. (1997). *Bartonella* spp. as Emerging Human Pathogens. // Clin Microbiol Rev. - V.10, - pp. 203-219. <https://doi.org/10.1128/cmr.10.2.203>.
- Angelakis, E., Raoult, D. (2014). Pathogenicity and Treatment of *bartonella* Infections. // Int. J. Antimicrob. Agents -V.44, -pp. 16-2. <https://doi.org/10.1016/j.ijantimicag.2014.04.006>.
- Bai, Y., Malania, L., Castillo, D.A., Moran, D., Boonmar, S., Chanlun, A., Suksawat, F., Maruyama, S., Knobel, D., Kosoy, M. (2013). Global Distribution of *Bartonella* Infections in Domestic Bovine and Characterization of *Bartonella bovis* Strains Using Multi-Locus Sequence Typing // PLoS One -V. 8, - pp. e80894. <https://doi.org/10.1371/journal.pone.0080894>.
- Ben-Tekaya, H., Gorvel, J.P., Dehio, C. (2013). *Bartonella* and *Brucella*– Weapons and Strategies for Stealth Attack // Cold Spring Harb. Perspect. Med.-V. 3, -pp. a010231. <https://doi.org/10.1101%2Fcshperspect.a010231>.
- Breitschwerdt, E.B. (2017). Bartonellosis, One Health and All Creatures Great and Small. // Vet. Dermatol. -V. 8, - Chapter 5.2, - pp. 111-121. <https://doi.org/10.1002/9781119278368.ch5.2>.
- Breitschwerdt, E.B., Greenberg, R., Maggi, R.G., Mozayani, B.R., Lewis, A., Bradley, J.M. (2019). *Bartonella henselae* Bloodstream Infection in a Boy with Pediatric Acute-Onset Neuropsychiatric Syndrome. // J. Cent. Nervous Syst. Dis. - V. 11 <https://doi.org/10.1177/1179573519832014>.
- Cheslock, M.A., Embers, M.E. (2019). Human Bartonellosis: An Underappreciated Public Health Problem? // Trop. Med. Infect. Dis. - V. 4, - p. 69. <https://doi.org/10.3390/tropicalmed4020069>.
- Chomel, B.B., Boulouis, H.J., Breitschwerdt, E.B. (2004). Cat Scratch Disease and Other Zoonotic *Bartonella* Infections. // J Am Vet Med Assoc. – V. 224, - pp. 1270–1279. <https://doi.org/10.2460/javma.2004.224.1270>.
- Chomel, B.B., Boulouis, H.J., Maruyama, S., Breitschwerdt, E.B. (2006). *Bartonella* spp. in Pets and Effect on Human Health // Emerg Infect Dis. –V. 12, - pp. 389-394. <https://doi.org/10.3201/eid1203.050931>.
- Chomel, B.B., Kasten, R.W., Williams, C., Wey, A.C., Henn, J.B., Maggi, R., Carrasco, S., Mazet, J., Boulouis, H.J., Maillard, R. (2009). *Bartonella* endocarditis: A Pathology Shared by Animal Reservoirs and Patients. // Ann. N. Y. Acad. Sci. –V. 1166, - pp. 120–126. <https://doi.org/10.1111/j.1749-6632.2009.04523.x>.
- Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I.S., Harrus, S. (2015). *Bartonella* Infection in Rodents and their Flea Ectoparasites: an Overview // Vector Borne Zoonotic Dis. – V. 15, - pp. 27-39. <https://doi.org/10.1089/vbz.2014.1606>.
- Harutyunyan, N., Balayan, M., Tsaturyan, V., Manvelyan, A., Pepoyan, A., Piceno, Y., Torok, T. (2014a). Effects of Probiotics on the Gut Microbiota Composition of Armenian Populations

- with Familial Mediterranean Fever Disease. International Conference “Trends in Microbiology and Microbial Biotechnology”. October 5-8, Yerevan, YSU press, - p. 3, isbn 978-5-8084-1895-0 https://www.researchgate.net/publication/267094941_EFFECTS_OF_PROBIOTICS_ON_THE_GUT_MICROBIOTA_COMPOSITION_OF_ARMENIAN_POPULATIONS_WITH_FAMILIAL_MEDITERRANEAN_FEVER_DISEASE.
13. Harutyunyan, N.A., Manvelyan, A.M., Balayan, M.H., Mirzabekyan, S.S., Malkhasyan, L.M., Pepoyan, A., Piceno, Y., Torok, T. (2013). Philochip™ Microarray Comparison of Sampling Methods Used for Gut Microbiota Investigation. “The 2nd International Scientific Conference of Young Researches on Biotechnology, General and Applied Microbiology, Chemistry, Biochemistry, Molecular Biology and Genetics, Environmental Protection”. October 1-4, Yerevan, Book of Abstracts, -pp. 83-84.
 14. Harutyunyan, N., Tsaturyan, V., Manvelyan, A., Balayan, M., Pepoyan, A., Piceno, Y., Torok, T. (2014b). Comparative Analysis of Bacterial Enumerations in Fecal Samples of Patients with Familial Mediterranean Fever Disease by Culture-Based and Phylochip Techniques. “3-rd International Conference on Clinical Microbiology and Microbial Genomics”. September 24-26, Valencia, Spain, -p. 57.
 15. Iannino, F., Salucci, S., Di Provvido, A., Paolini, A., Ruggieri, E. (2018). *Bartonella* Infections in Humans, Dogs and Cats. // Vet. Ital. –V. 54, -pp. 63–72. <https://doi.org/10.12834/vetit.398.1883.2>.
 16. Kalogeropoulos, D., Asproudis, I., Stefanidou, M., Moschos, M.M., Mentis, A., Malamos, K., Kalogeropoulos, C. (2019). *Bartonella henselae*- and *quintana*-associated uveitis: A Case Series and Approach of a Potentially Severe Disease with a Broad Spectrum of Ocular Manifestations. // Int. Ophthalmol. - V. 39, - pp. 2505-2515. <https://doi.org/10.1007/s10792-019-01096-7>.
 17. Kosoy, M., Bai, Y., Sheff, K., Morway, C., Baggett, H., Maloney, S.A., Boonmar, S., Bhengsi, S., Dowell, S.F., Sidthirassr, A., Lerdtusnee, K., Richardson, J., Peruski, L.F. (2010). Identification of *Bartonella* Infections in Febrile Human Patients from Thailand and their Potential Animal Reservoirs. // Am J Trop Med Hyg. – V. 82, - pp. 1140–1145.
 18. Kosoy, M., Mckee, C., Albayrak, L., Fofanov, Y. (2018). Genotyping of *Bartonella* Bacteria and their Animal Hosts: Current Status and Perspectives. // Parasitology. - V. 145, - pp. 543-562. <https://doi.org/10.1017/s0031182017001263>.
 19. Kosoy, M., Murray, M., Gilmore, R.D., Jr., Bai, Y., Gage, K.L. (2003). *Bartonella* Strains from Ground Squirrels are Identical to *Bartonella washoensis* Isolated from a Human Patient. // J Clin Microbiol. –V. 41, - pp. 645–650. <https://doi.org/10.1128/JCM.41.2.645-650.2003>.
 20. Malania, L., Bai, Y., Osikowicz, L.M., Tsertsvadze, N., Katsitadze, G., Imnadze, P., Kosoy, M. (2016). Prevalence and Diversity of *Bartonella* Species in Rodents from Georgia (Caucasus). - V. 95, - pp. 466–471. <https://doi.org/10.4269/ajtmh.16-0041>.
 21. Piceno, Y.M., Harutyunyan, N., Balayan, M., Tsaturyan, V., Manvelyan, A., Pepoyan, A., Torok, T. (2013). Effects of Probiotics on the Gut Microbiota of Armenian Populations with Familial Mediterranean Fever Using PhyloChip™ and Culture-based Analyses. “International Scientific Conference on Probiotics and Prebiotics”. June 11 – 13, Kosice, Slovakia, Book of Abstracts, - pp. 37-38. https://researchgate.net/publication/261873152_Effects_of_probiotics_on_the_gut_microbiota_of_Armenian_populations_with_Familial_Mediterranean_Fever_using_PhyloChip_and_culture-based_analyses.
 22. Pepoyan, A., Balayan, M., Manvelyan, A., Galstyan, L. (2018). Probiotic *Lactobacillus acidophilus* Strain INMIA 9602 Er 317/402 Administration Reduces the Numbers of *Candida Albicans* and Abundance of Enterobacteria in the Gut Microbiota of Familial Mediterranean Fever Patients. // Frontiers in Immunology. – V. 9. <https://doi.org/10.3389/fimmu.2018.01426>.
 23. Pepoyan, A.Z., Balayan, M.A., Harutyunyan, N.A., Grigoryan, A.G., Tsaturyan, V.V., Manvelyan, A.M., Dilanyan, E., Pitseno, I., Torok, T. (2015a). Antibiotic Resistance of *Escherichia Coli* of the Intestinal Microbiota in Patients with Familial Mediterranean Fever. // Clin Med (Mosk). – V. 93, - pp. 37-39. (in Russian) https://researchgate.net/publication/283638301_Antibiotikorezistentnost_Escherichia_coli_kisechnoj_mikrobioty_u_bolnyh_semejnoj_sredizemnomorskoy_lihoradkoj.
 24. Pepoyan, A., Balayan, M., Harutyunyan, N., Manvelyan, A., Malkhasyan, L., Mirzabekyan, S., Isajanyan, M. (2014a). Placebo Effect in Familial Mediterranean Disease. “5th ASM Conference on Beneficial Microbes”. September 27 – 30, Washington, DC, - pp. 87. <https://researchgate.net/>

[publication/267094739 PLACEBO EFFECT IN FAMILIAL MEDITERRANEAN FEVER DISEASE.](#)

25. Pepoyan, A., Harutyunyan, N., Grigorian, A., Tsaturyan, V., Manvelyan, A., Dilanyan, E., Balayan, M., Torok, T. (2015b). The Certain Clinical Characteristics of Blood in Patients with Familial Mediterranean Fever of Armenian Population. *Klinicheskaia Laboratornaia Diagnostika* // - V. 60, - pp. 46-7. (in Russian) https://researchgate.net/publication/349075309_scopuspng.
26. Pepoyan, A., Harutyunyan, N., Pepoyan, E., Tsaturyan, V., Torok, T. (2019). Relationship between the Numbers of *Candida albicans* and Abundance of *Helicobacter spp.* in the Gut Microbiota of Familial Mediterranean Fever Patients. // *Helicobacter*. - V. 24(S1), - pp. e12647 (70). <https://doi.org/10.1111/hel.12647>.
27. Pepoyan, A., Harutyunyan, N., Tsaturyan, V. (2014b). Comparative Analysis of Bacterial Enumerations in Fecal Samples of Patients with Familial Mediterranean Fever Disease by Culture-Based and Phylochip Techniques. *Clin Microbiol.* – V. 3, - p. 57.
28. Pepoyan, A.Z., Pepoyan, E.S., Galstyan, L., Harutyunyan, N.A., Tsaturyan, V.V., Torok, T., Ermakov, A.M, Popov, I.V., Weeks, R., Chikindas, M.L. (2021). The Effect of Immunobiotic/ Psychobiotic *Lactobacillus acidophilus* Strain INMIA 9602 Er 317/402 Narine on Gut *Prevotella* in Familial Mediterranean Fever: Gender-Associated Effects. // *Probiotics & Antimicro. Prot.* – V. 13, - pp. 1306-1315. <https://doi.org/10.1007/s12602-021-09779-3>.
29. Petriková, K., Halánová, M., Babinská, I. (2021). Seroprevalence of *Bartonella henselae* and *Bartonella quintana* Infection and Impact of Related Risk Factors in People from Eastern Slovakia // *Pathogens*. - V. 10, - pp. 1261. Published 2021 Sep 29. <https://doi.org/10.3390/pathogens10101261>.
30. Razgūnaitė, M., Lipatova, I., Paulauskas, A., Karvelienė, B., Riškevičienė, V., Radzijeuskaja, J. (2021). *Bartonella* Infections in Cats and Cat Fleas in Lithuania. // *Pathogens*. – V. 10, - pp. 1209. <https://doi.org/10.3390/pathogens10091209>.
31. Ying, B., Kosoy, M.Y., Maupin, G.O., Tsuchiya, K.R., Gage, K.L. (2002). Genetic and Ecologic Characteristics of *Bartonella* Communities in Rodents in Southern China. // *Am J Trop Med Hyg.* – V. 66, -pp.622–627. <https://doi.org/10.4269/ajtmh.2002.66.622>.

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